Study of Physiological and Biochemical Properties of *Candida albicans* Strains Causing Various Diseases

YVONNE SVOBODOVÁ and L. DROBNICA

Laboratory for Mycological Research of the Dermatological Clinic, Faculty of Medicine, Komenský University, Bratislava, and Department of Technical Microbiology and Biochemistry, Faculty of Chemistry, Slovak Technical College, Bratislava

Received September 8, 1960

In the course of clinical and laboratory examinations the genus *Candida* is often identified as the causative agent of various yeast-caused ailments. The most frequent pathogenic factor within that genus is *Candida albicans* (Pospíšil, 1957). Other species of this genus occur less frequently as pathogens (Wikler, 1942; Polsays, 1941; Pospíšil & Pokorný, 1957). Rieth (1958) points out the very frequent occurence of *Candida albicans* in the oral cavity, in gastric juice, in the stool, in the vulva and vagina as well as in skin scales and nails. Rieth isolated 2114 strains of the *Candida* genus, *Candida albicans* being by far the most frequent (1111 strains) followed by *Candida parapsilosis* (342 strains) and others. Helms (1956) analyzed the sputum of 205 untreated patients with respiratory diseases and found *Candida albicans* in 77% cases. Pillich and Svobodová (1958) who examined yeast-like microorganisms in the respiratory tract of untreated patients diagnosed 48 cases affected by *Candida albicans*.

As criteria for the identification of the species and for determining the particular variety we employ physiological and morphological features compiled in the most widely used work on taxonomy by Lodder and Kreger van Rij (1952). These properties usually comprise the botanical characteristic of the species.

The question still remains open whether any differences exist in the morphology and the metabolism of strains of *Candida albicans* isolated in various pathological conditions, whether these differences are closely connected with the corresponding pathogenicity of strains and, finally, whether any type properties can be attributed to strains isolated during a given pathological process, thus suggesting the capacity of causing predominantly the given type of disease. By solving these problems the question of pathogenicity of various *Candida* species can be elucidated.

In the present work attention was paid to strains of *Candida albicans* bringing about pulmonary and vaginal candidosis, yeast onychomycosis and superficial candidosis.

The experiments cited here represent attempts at investigating the biochemical and physiological differences existing between pathogenic strains isolated from typical clinical cases and may contribute to our understanding the physiology of *Candida albicans*.

In this connection it is of importance to mention especially the data on the vitamin requirements of the pathogenic *Candida albicans* strains investigated; this problem was also touched upon by other authors (Kaden, 1955; Karasevich, 1957; Drouhet & Vieu, 1957; Jelinov & Zaikina, 1960).

MATERIALS AND METHODS

Isolation of strains. We used strains from collections and freshly isolated from patients with the following typical pathological processes:

Strain A is a collection strain maintained for 3 years at the Department of

Technical Microbiology and Biochemistry, Slovak Technical College in Bratislava (originally isolated from the lungs of an infant after death). Strain B derives from yeast onychomycosis lasting for 2 years. The material was removed after injury to the nail. Strains C originates from pulmonary candidosis of two years standing. Strain D caused superficial candidosis lasting for 2 years. Strain E was isolated from vaginal candidosis of three years' duration. The isolation was carried out from untreated patients over a period of two months and pure cultures were maintained on Sabouraud's glucose agar at laboratory temperature. Pure cultures were transferred through acid Raulin's solution. All strains were identified as *Candida albicans* according to criteria of Lodder and Kreger van Rij (1952).

Morphological characterization of cells and colonies. Cells were characterized morphologically as described by Kocková-Kratochvílová and Drobnica (1957). Liquid Sabouraud's glucose medium was used (100 ml. medium in 250 ml. flasks). The measurement was carried out after three days of incubation at 37° C. Cells in pseudomycelia were not taken into account. The size of a sample was 100 cells. The appearance and shape of colonies was evaluated after 30 days of growth at 37° C on Sabouraud's glucose medium.

Preliminary demonstration of pathogenicity. A loop from each culture was transferred to test-tubes containing 5 ml. physiological saline and the suspension formed was injected into a rabbits' ear. Perished animals were autopsied and their organs examined by a cultivation test and histologically.

Growth and vitamin requirements. For determining the growth curves and vitamin requirements the cultures were transferred three times on the corresponding medium. In all strains the propagation on liquid Sabouraud's glucose medium and on a synthetic medium with vitamins was examined. The synthetic medium (1000 ml) contained: glucose 50 g.; $(NH_4)_2SO_4$ 4 g.; KCl 4.5 g.; 10 ml. solution of biogenic elements (100 ml. of stock solution contains $CaCl_2 . H_2O$ 12.5 g.; $MgSO_4 . 7H_2O$ 12.5 g.; $FeCl_3 . 6H_2O$ 0.42 g. $MnSO_4$ 0.25 g.) and 10 ml. 0.2 M phosphate buffer of pH 6.5. The synthetic medium with vitamins was prepared by adding 1 ml. solution of vitamins containing in 100 ml.: mesoinositol 25 mg.; pyridoxine 5 mg.; calcium pantothenate 10 mg.; thiamine 5 mg.; nicotinic acid 5 mg.; d-biotin 0.005 mg. The solution was sterilized at 120° C for 25 min. After sterilization the pH was 6.1 (Drobnica, 1960).

In investigating the requirements of strains with regard to vitamins the synthetic medium was modified in such a way that one of the 6 vitamins was omitted or else all of them were left out (synthetic medium without vitamins).

Determination of growth curves. The propagation of *Candida albicans* strains was examined as in an earlier paper (Nemec *et al.* 1958). Parallel samples were always used. In order to determine the growth curves of the strains investigated under conditions of aeration, 500 ml. flasks with 100 ml. Sabouraud's glucose medium were inoculated with 0.4 ml. of 48-hour-old cultures. Cultivation proceeded on a reciprocal shaker at 37° C and 90 strokes/min. At given time intervals 5 ml. samples were removed. The samples were centrifuged at 3000 rev./min. for 20 min. and the yeast cells were resuspended in a known volume of physiological saline so that the suspension turbidity lay within the calibration curve. The corresponding number of cells per ml. was multiplied by the appropriate dilution factor.

Determination of RQ. The respiratory quotients were determined in strains grown either under static conditions or under conditions of intense aeration. The static cultivation was carried out in 100 ml. Sabouraud's glucose medium in 250 ml. flasks at 30° C for 48 hrs. (logarithmic phase). The cells were then centrifuged, washed three times and

resuspended in physiological saline. The suspensions were made to the same turbidity (% absorption = 70, 5 ml. cuvette) and placed for 48 hours into a refrigerator. The aerated cultures were cultivated on a rotary shaker for 24 hrs. (1000 ml. flasks with 250 ml. Sabouraud's glucose medium) at 30° C. The RQ was determined by direct Warburg's method in the presence of glucose as energy-yielding substrate ($V_k = 3.4$, inoculum = 1 ml. suspension, side-arm: 1 ml. 3% glucose solution; central well: 0.2 ml 30% KOH or water and a folded piece of filter paper; T = 25° C, number of strokes 120/min., amplitude 60°).

RESULTS AND DISCUSSION

Table 1 summarizes the results of the mathematical and statistical treatment of cell morphology of the individual *Candida albicans* strains. From the point of view of morphology all strains are characterized by round cells with a high correlation coefficient which points to a morphological uniformity of cells. After a 3-day cultivation no formation of pseudomycelium could be observed. More marked differences existed between the individual strains with regard to the shape and appearance of giant colonies (Figs. 1—5).

Preliminary experiments revealed pathogenicity for animals. A rabbit infected i. v. (into the ear) with the C strain died after 28 hrs., with the E strain after 48 hrs., with the B strain after 62 hrs., with the D strain after 7 days and with the A strain after 10 days. All dead rabbits displayed macroscopic changes in the kidneys: swelling, marked congestion and the entire surface contained minute abscesses of poppy-seed size (Fig. 6). Histological preparations from rabbit kidneys showed yeast-like cells with blastospores (Fig. 7, 7a). Yeast-like cells were reisolated from the kidney abscesses.

Fig. 8 shows the course of multiplication of *Candida albicans* strains under investigation, both in a medium with organic sources of nitrogen (Sabouraud's glucose medium) and in a synthetic medium with and without vitamins, containing a mineral source of nitrogen. The growth curves thus permit conclusions to be drawn as to the requirements of each strain for the source of nitrogen, presence of vitamins, as well as the role of aeration. The similarity in the character of growth curves of strain C (pulmonary candidosis) and strain E (vaginal candidosis) is especially marked. At the same time, the course of multiplication on Sabouraud's glucose medium and on the vitamin-containing medium does not reflect any difference as exist between these two cultivation media. The omission of vitamins from the synthetic medium is

Table 1. Cell size of 5 strains of *Candida albicans* after 3-day cultivation on Sabouraud's glucose medium

Strain	Origin	Disease	Mean length (a) $\bar{x} \pm s_{\bar{x}}$	Mean width (μ) $\bar{y} \pm s_{\bar{y}}$	Correlation coeficient r	Length: width ratio x : y
A		Collection strain Pn 10	7.86 ± 0.179	7.6 ± 0.172	0.94	1.03
B	Nails	Yeast onychomycosis	8.51 ± 0.194	8.24 ± 0.182	0.94	1.03
C	Sputum	Pulmonary candidosis	7.06 ± 0.159	7.06 ± 0.147	0.97	1.02
D	Skin scale	Superficial candidosis	6.13 ± 0.179	8.92 ± 0.190	0.96	1.03
E	Vagina	Vaginal candidosis	6.81 ± 0.155	6.8 ± 0.142	0.91	1.00

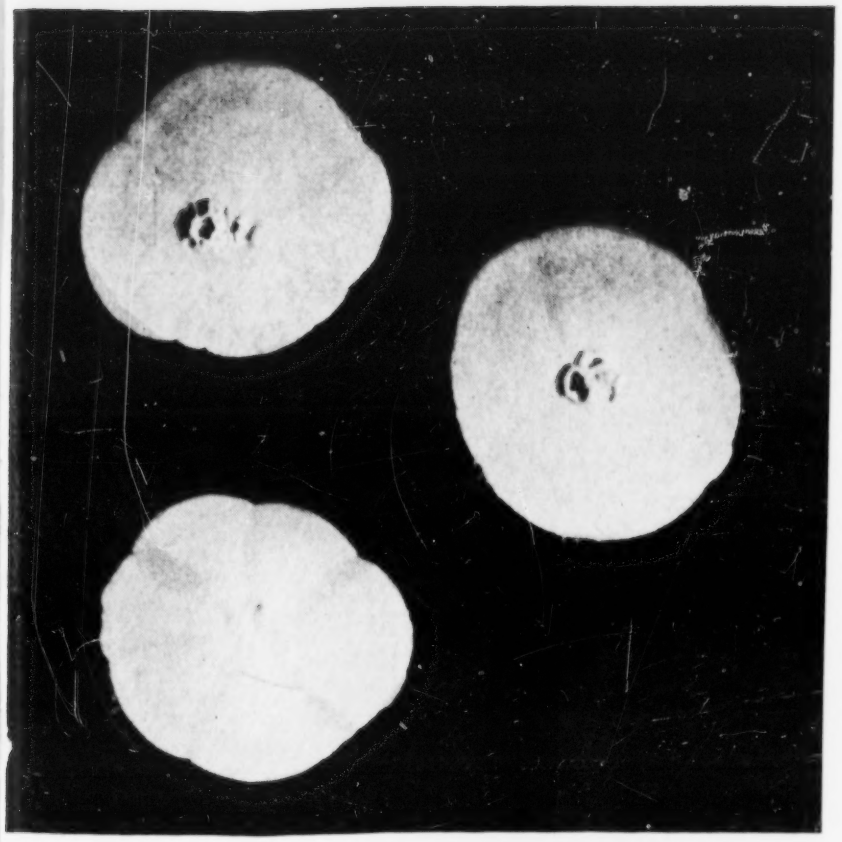


Fig. 1. Vaginal candidosis (from vagina).

Fig. 2. Yeast onychomyosis (from finger-nails).

Fig. 3. Pulmonary candidosis (from sputum).

Giant colonies of strains of Candida albicans isolated from pathological processes of different localization. Giant colonies after 30 days of growth on Sabouraud's glucose medium (enlarged approximately 1.3 times).

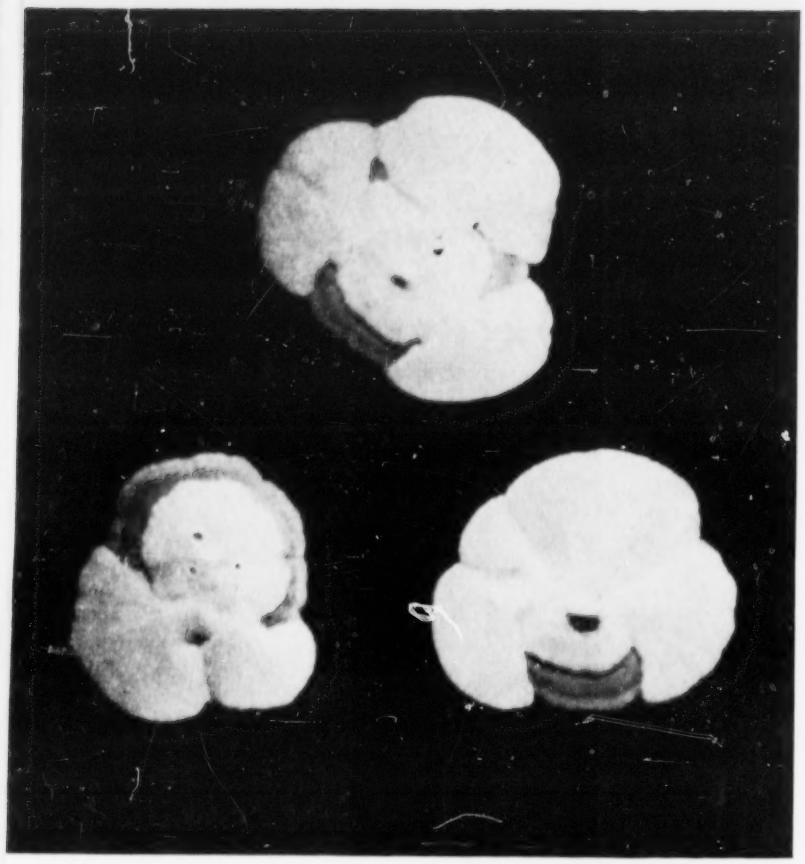


Fig. 4. Superficial candidosis (from skin-scale).

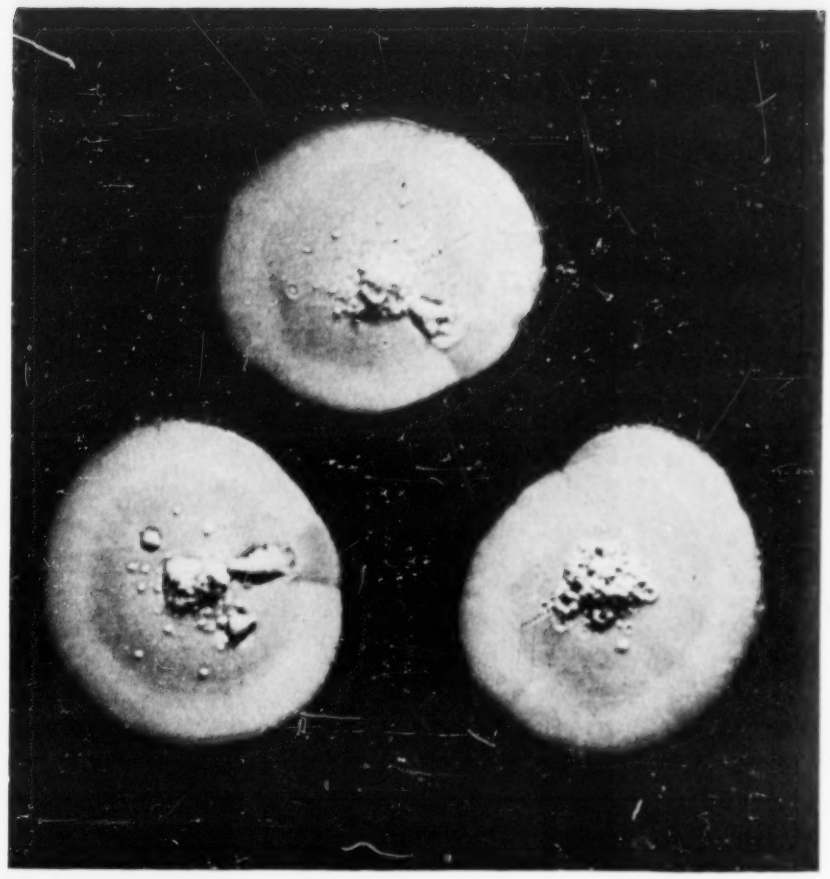


Fig. 5. Collection strain. Colour of colonies greyish in strain B, cream in others.

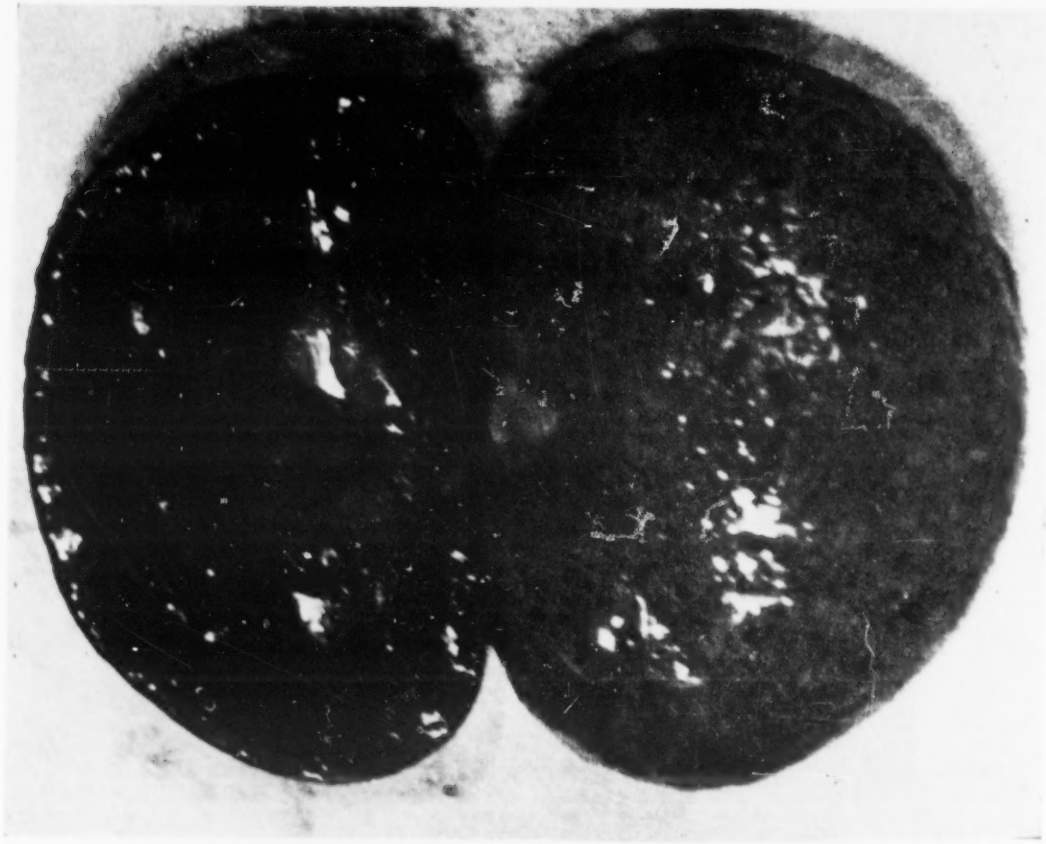


Fig. 6. Clinical appearance of rabbit kidney after intravenous injection of 0.5 ml. suspension of *Candida albicans*, isolated from sputum in a patient with pulmonary candidosis. The surface of the kidney is shiny, covered with minute, discrete abscesses of poppy-seed size.

Giant colonies of strains of Candida albicans isolated from pathological processes of different localization. Giant colonies after 30 days of growth on Sabouraud's glucose medium (enlarged approximately 1.3 times).

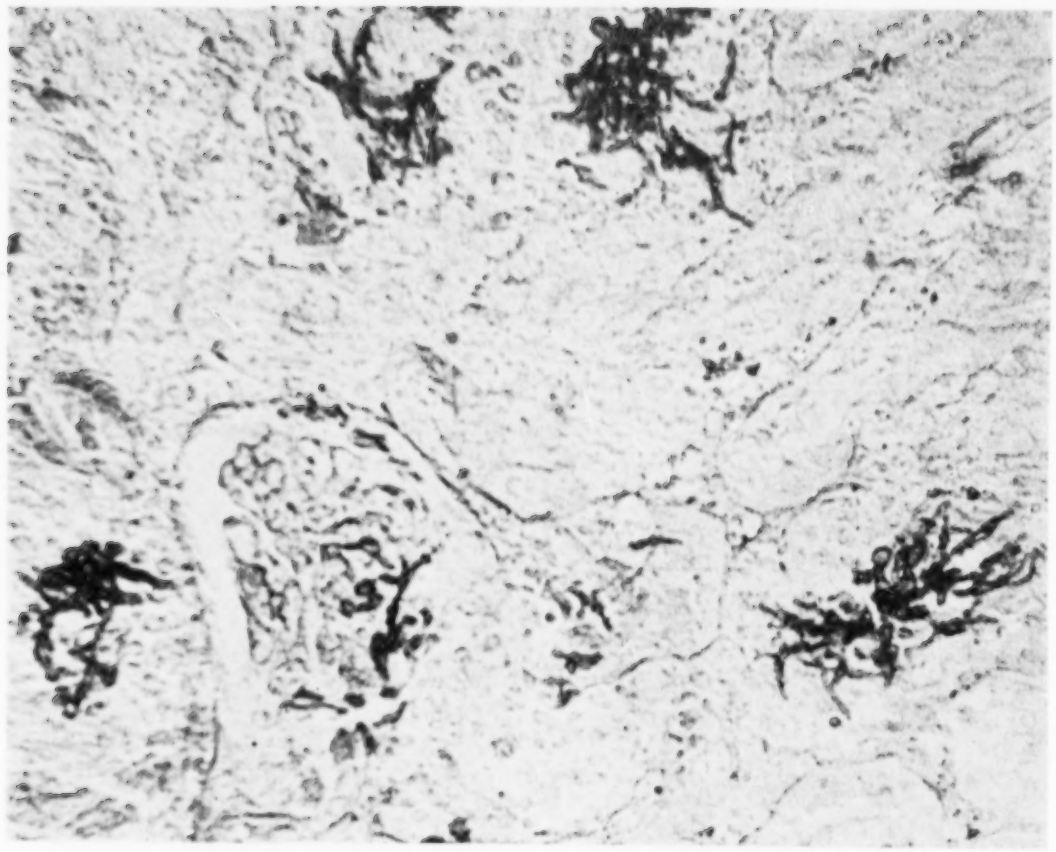


Fig. 7. A histological section through rabbit kidney infected with *Candida albicans* strain reveals clusters of yeast-like cells with blastospores and rich pseudomycelium formation, localized mostly in the glomeruli. Stained with PAS.

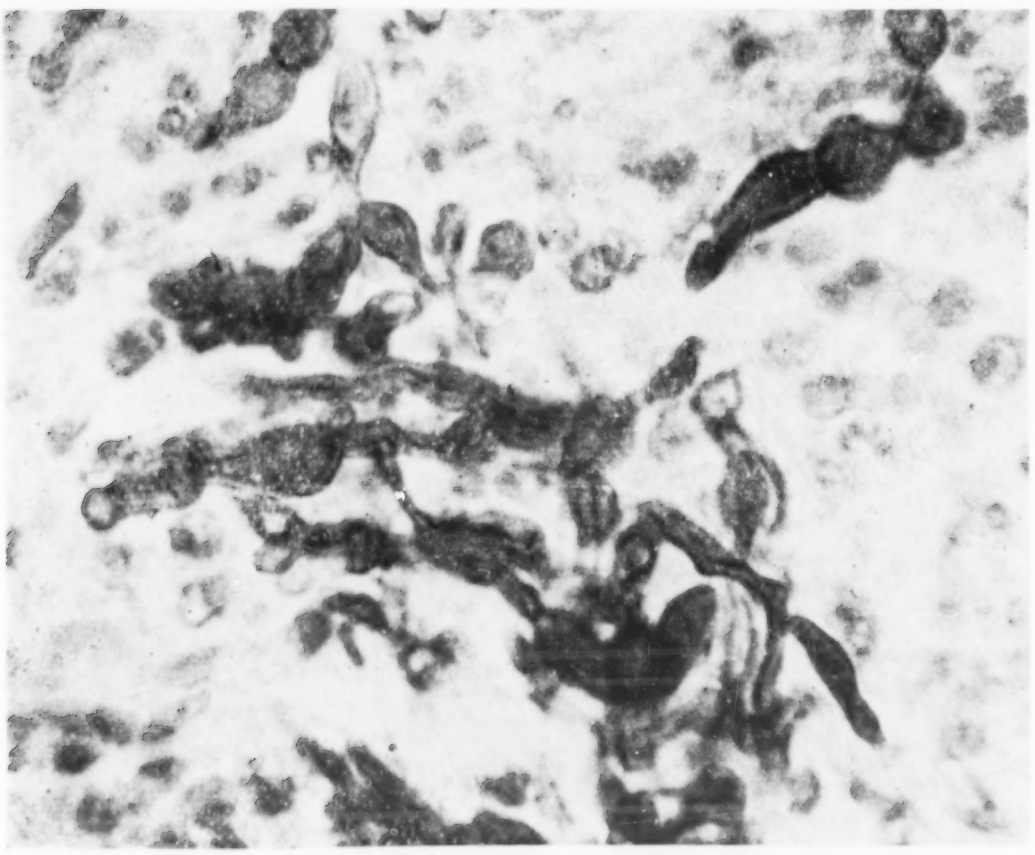
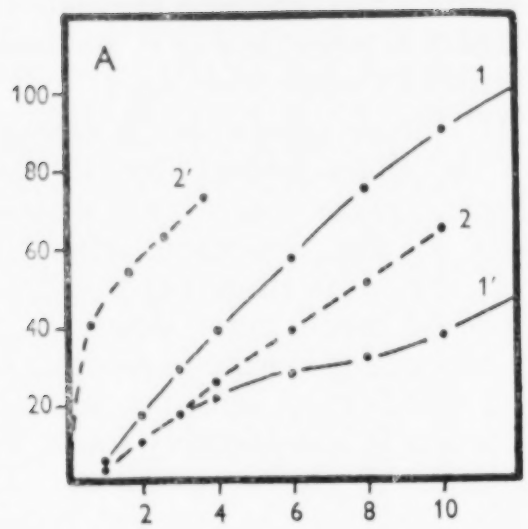
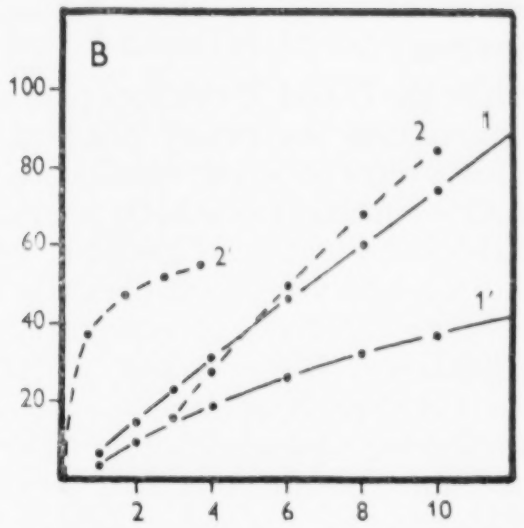
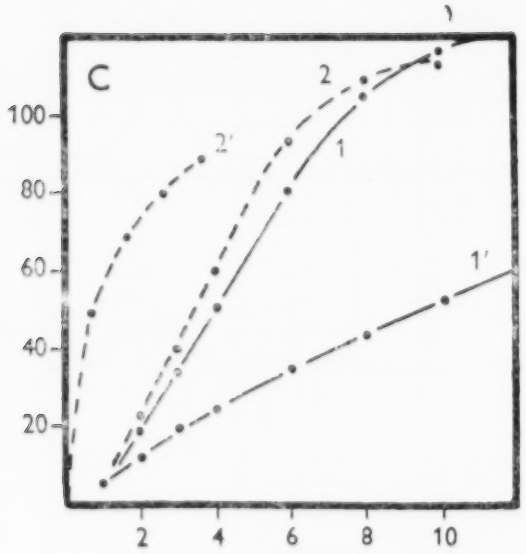


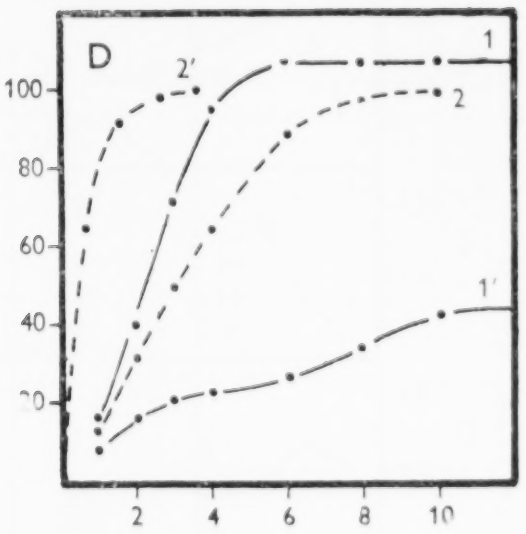
Fig. 7a. A detail of histological section through rabbit kidney suffering from experimentally induced candidosis.

Fig.









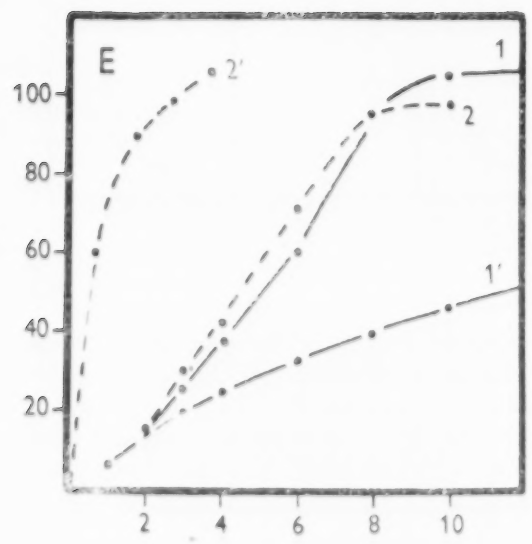


Fig. 8. Multiplication of strains of *Candida albicans* under different cultivation conditions. 1 — synthetic medium with vitamins; 1' — synthetic medium without vitamins; 2 — Sabouraud's glucose medium; 1, 1,2 — static culture (growth in test-tubes). 2' — Sabouraud's glucose medium — shaken culture (the cell count shown should be multiplied by ten). Cultivation at 37° C. Inoculum 1,1—0.05 ml. of 48-hour-old cultures on synthetic medium with vitamins, in 2—0.05 ml. and in 2—0.4 ml. 48-hour-old cultures from Sabouraud's glucose medium. A — collection strain, B — finger-nail, C — lungs, D — skin, E — vagina. x — time (days), y — number of cells $\times 10^6$/ml.

however reflected in the retarded multiplication.

While the C and E cultures pass over to the maximum stationary phase as early as after 8 days of static cultivation in the vitamin-containing synthetic medium and in Sabouraud's glucose medium, cultures B and A retain their multiplication ability even after 12 days of cultivation. The multiplication is slower and a difference exists between Sabouraud's glucose medium and the vitamin-containing synthetic medium. In culture B the growth curve in Sabouraud's glucose medium points to its higher suitability for multiplication. The opposite is the case with the collection strain, which is possibly due to its numerous transfers through wort agar.

The D culture grown in Sabouraud's medium is characterized by a sharp logarithmic phase and a clear-cut transition to the maximum stationary phase. At the same time, the multiplication proceeds much more slowly in the vitamin-containing synthetic medium and the total number of cells per ml. after multiplication is considerably lower than in Sabouraud's glucose medium. This strain also displays the greatest requirement for vitamins.

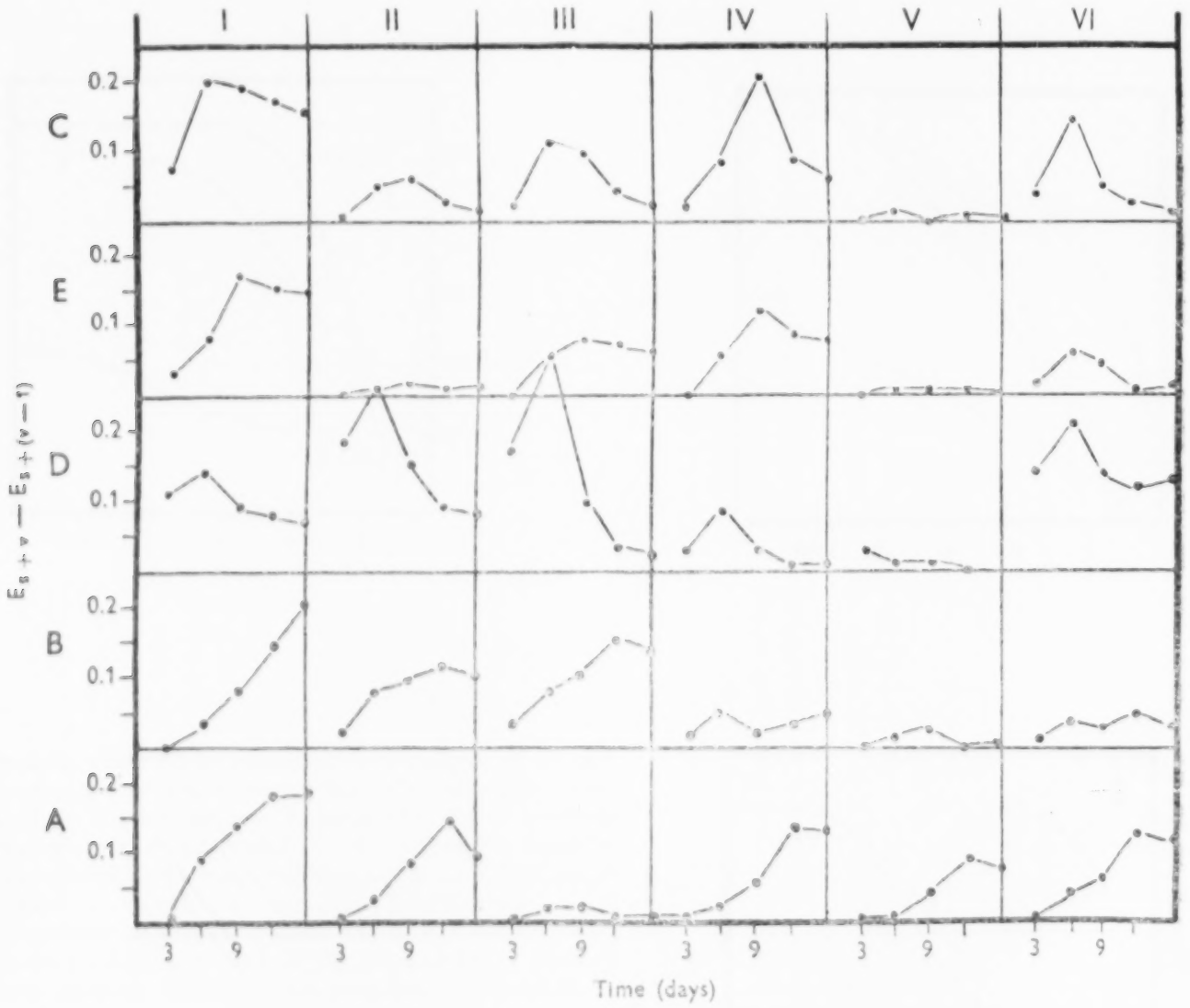


Fig. 9. The effect of lack of individual vitamins on the reproduction of strains of *Candida albicans*. (The values are calculated on the basis of data from Tables I—5). E_{S+V} — extinction characterizing reproduction in a synthetic medium with 6 vitamins, E_{S+V-1} — extinction when one vitamin is omitted in the corresponding column. A—E — strains of *Candida albicans*. I — biotin, II — nicotinic acid, III — thiamine, IV — pyridoxine, V — pantothenate, VI — inositol.

In the present paper we attempt to define more clearly the requirements of the individual strains for vitamins. One of the six vitamins present in the complete medium was always omitted. Fig. 9 summarizes the data on the course of multiplication of the strains tested in a synthetic medium. The following conclusions can be drawn: (1) The omission of biotin from the synthetic medium brings about a retardation of multiplication of all strains. Judging from the character of the growth curves this retardation is displayed in a lower number of cells in the maximum stationary phase as compared with the control. (2) With the exception of strain A the strains tested do not require pantothenate. The opposite is true for thiamine (3). It was shown that strains C and E could grow practically without nicotinic acid (4). Of all the strains tested only the onychomycosis strain B did not require pyridoxine and inositol. (5) Strains C and E are identical in their vitamin requirements.

The character of the curves shown in Fig. 9 permits the conclusion to be drawn that the lack of a given vitamin results in a prolongation of the logarithmic phase of the growth curve (as compared with the control) or else is displayed in a decreased number of cells per ml. during the maximum stationary phase. The first case is exemplified by the lack of biotin in cultures A and B while the second is exemplified by multiplication in a me-

Table 2. Respiration and respiration quotients of static cultures of *Candida albicans* strains (with glucose)

Time in hrs.	A			B			C		
	X_{O_2}	RQ	$X_{O_2}Er$	X_{O_2}	RQ	$X_{O_2}Er$	X_{O_2}	RQ	$X_{O_2}Er$
1	136	1.15	17	72	1.10	48	66	1.78	15
2	270	1.14	40	260	1.07	72	166	1.46	20
3	476	1.17	83	305	1.10	123	300	1.28	26
	D			E					
1	52	1.13	5	45	1.84	10			
2	100	1.16	20	125	1.44	34			
3	261	1.17	56	286	1.31	65			

$X_{O_2}Er$ = consumption of oxygen corresponding to endogenous respiration

Table 3. Respiration and respiration quotients of shaken cultures of *Candida albicans* strains (with glucose)

Time in hrs.	A			B			C		
	X_{O_2}	RQ	$X_{O_2}Er$	X_{O_2}	RQ	$X_{O_2}Er$	X_{O_2}	RQ	$X_{O_2}Er$
1	86	4.0	58	30	3.6	26	41	2.5	20
2	186	2.15	120	98	1.98	72	118	2.5	49
3	345	1.97	283	241	1.37	180	253	2.3	105
	D			E					
1	164	1.14	120	64	2.84	26			
2	380	1.08	228	124	2.90	57			
3	—	—	—	—	—	—			

3718

dium without thiamine or pyridoxine in culture D.

By estimating respiratory quotients in static and shaken cultures of the various strains we intended to verify whether the individual strains will differ in the character of their energy metabolism or in the degree of oxidative degradation of glucose (fermentative vs. respiratory type). On the basis of the growth curves it can be concluded that all the strains grown under aeration reach the logarithmic phase of growth after 24 hrs. of cultivation while those grown statically reach it after 48 hrs. Therefore cultivation was interrupted after this period, the cells were washed and maintained at 3° C for 48 hrs. (to decrease endogenous respiration).

The results summarized in Table 2 and 3 suggest that there are no marked differences in the character of energy metabolism of the strains investigated (RQ values on glucose) and that it is unlikely that possible type differences can be associated with differences (genetically fixed in the individual strains) in their requirement for oxygen.

SUMMARY

Morphological and physiological properties of 5 strains of *Candida albicans* were studied, four of them causing a typical disease (A — collection strain, B — onychomycosis, C — pulmonary candidosis, D — superficial candidosis, E — vaginal candidosis).

On evaluating the morphology of cells of cultures from liquid Sabouraud's glucose medium, differences in cell size were found. The individual strains differ in the morphology of their colonies.

On the basis of estimating the growth on Sabouraud's glucose medium and on a synthetic medium, and of the vitamin requirements, the striking similarity of strains C and E became apparent, the two causing pulmonary and vaginal candidosis, respectively.

The omission of biotin from the synthetic medium in the presence of other vitamins brought about a retardation of multiplication in all strains. With the exception of the collection strain A the reproduction of other strains under the given conditions was not stimulated by pantothenate. Only with strain B causing onychomycosis did the omission of pyridoxine and inositol not affect the course of reproduction.

On the basis of RQ values the assumption can be ruled out that possible type differences between individual strains can consist in a different requirement for oxygen that might be permanently fixed in the individual strains.

References

Drobnica, L.: *Adaptation of yeast and bacteria to antimicrobial substances during continuous-flow cultivation*. Fol. microbiol. 5 : 257, 1960.

Droubet, E., Vieu, M.: *Facteurs vitaminiques de croissance des Candida*. Ann. Inst. Pasteur 6 : 825, 1957.

Helms, P.: *The occurrence of C. albicans in sputum in Denmark*. J. clin. Path. 9 : 327, 1956.

Jelinov, N. P., Zaĭkina, N. A.: *The effect of vitamins and hormones of the growth of yeast-like organisms of the genus Candida*. Mikolog. issled., Leningrad 1960 (in Russian).

Kaden, R.: *Vitamine und Pilzwachstum*. Arch. Dermatolog. Syphilis 200 : 256, 1955.

Kocková-Kratochvílová, A., Drobnica, L.: *Roztřídění cizných kvasinek*. Preslia 29 : 269, 1957.

Karasevich, Yu. N.: *Vitamin requirements of some strains of fodder yeast*. Mikrobiologiya 26 : 106, 1957 (Карасевич, Ю. Н.: Микробиология 26 : 106, 1957).

Lodder, J., Kreger van Rij, N. J. W.: *The Yeasts*. North-Holland Publ. Co., Amsterdam 1952.

Nemec, P., Drobnica, L., Antoš, L., Kristian, P., Hulka, A.: *Antimikrobiálna účinnosť syntetických izotiokyanátov*. 1958. II. Niektoré aromatické ITK. Biol. práce Slov. akad. věd IV/9. Vyd. Slov. akad. věd, Bratislava 1958.

Pillich, J., Svobodová, Y.: *Kvasinkové mikroorganizmy v dýchacích cestách*. Rozhl. v tuberkulóze a nemocech plicnych (in press).

Polsays, S. H.: *Final report on the identification of the organism of the previously reported case of subacute endocarditis and systemic mycosis (Monilia)*. J. Am. med. Assoc. 117 : 1533, 1941.

Pospíšil, L., Pokorný, J.: *Serologické sledování kandidových flór*. Čsl. dermatologie 32 : 4, 1957.

Pospíšil, L.: *Serologie kandidových infekcí*. Thesis, Faculty of Medicine, Brno 1957.

Rieth, H.: *Untersuchungen zur Hefediagnostik in der Dermatologie*. Arch. klin. exp. Dermatologie 207 : 413, 1958.

Wikler, A.: J. Am. Med. Assoc. 119 : 333. 1942, cited from Carlson, S.: *Über die pathogenetische Bedeutung und Ausbreitung der Hefen unter der Antibiotika-Therapie*. Zbl. Bakt. Ref. 160 : 173, 1956.

ИЗУЧЕНИЕ ФИЗИОЛОГИЧЕСКИХ И БИОХИМИЧЕСКИХ СВОЙСТВ ШТАММОВ CANDIDA ALBICANS, ВЫЗЫВАЮЩИХ РАЗЛИЧНЫЕ ЗАБОЛЕВАНИЯ

И. Свободова и Л. Дробница

Изучались морфологические и физиологические особенности 5 штаммов Candida albicans, 4 из которых вызывали типичные заболевания (А — музейный штамм, В — штамм, вызывающий онихомикоз, С — легочный кандидоз, Е — вагинальный кандидоз).

Были обнаружены различия в размерах клеток из культур в жидкой среде с глюкозой по Saboraud. Отдельные штаммы отличаются друг от друга по морфологии колоний.

На основе оценки роста на среде с глюкозой по Saboraud и на синтетической среде, а также требований к витаминам, обнаружилось выраженное сходство между штаммами С и Е, вызывающими легочный и влагалищный кандидоз.

В синтетической среде без биотина, но в присутствии остальных витаминов, наблюдалось замедление размножения всех штаммов. Кроме музейного штамма А, размножение остальных штаммов при данных условиях не стимулировалось пантотенатом. Отсутствие в среде пиридоксина и инозитола не влияло на процесс размножения только у штамма В, вызывающего онихомикоз.

На основании RQ пришлось отказаться от предположения, что встречающиеся иногда типовые различия между отдельными штаммами обусловливаются различными и у отдельных штаммов устойчиво закрепленными требованиями к кислороду.